

# Qualitative Phytochemical Analysis and Antibacterial Activity Evaluation of Indian *Terminalia* spp. Against the Pharyngitis causing pathogen *Streptococcus pyogenes*

Mitchell Henry Wright,<sup>1</sup> Megan Sarah Jean Arnold,<sup>2</sup> Cameron Jay Lee,<sup>1</sup> Reece Courtney,<sup>1,3</sup> Anthony Carlson Greene,<sup>1</sup> Ian Edwin Cock<sup>1,3\*</sup>

<sup>1</sup>School of Natural Sciences, Nathan Campus, Griffith University, 170 Kessels Rd, Nathan, Queensland 4111, AUSTRALIA.

<sup>2</sup>Eskitis Institute for Drug Discovery, Griffith University, Queensland, Australia, 46 Don Young Rd, Nathan, Queensland 4111, AUSTRALIA.

<sup>3</sup>Environmental Futures Research Institute, Nathan Campus, Griffith University, 170 Kessels Rd, Nathan, Queensland 4111, AUSTRALIA.

## ABSTRACT

**Introduction:** *Streptococcus pyogenes* is a gram-positive, pathogenic bacterium which causes a variety of diseases including streptococcal pharyngitis, impetigo and rheumatic heart disease, depending on which tissue it infects. Many *Terminalia* spp. have documented therapeutic properties as general antiseptics, inhibiting the growth of a wide variety of bacterial species. **Methods:** Solvent extracts were prepared using Indian *Terminalia* spp. with documented ethnobotanical usage to treat bacterial infections, or published antibacterial activity. The extracts were investigated by disc diffusion assay for the ability to inhibit the growth of a clinical strain of *S. pyogenes*. Their MIC values were determined to quantify and compare their efficacies. Toxicity was determined using the *Artemia franciscana* nauplii bioassay. **Results:** *T. arjuna*, *T. catappa* and *T. chebula* methanolic and ethyl acetate extracts displayed potent antibacterial activity in the disc diffusion assay against *S. pyogenes*. The *T. catappa* and *T. chebula* ethyl acetate extracts were particularly potent, with MIC values of 225 and 205 µg/mL respectively. All methanolic extracts were also potent growth inhibitors with MIC values of 268 µg/mL (*T. arjuna* methanolic extract), 425 µg/mL (*T. catappa* methanolic extract) and 300 µg/mL (*T. chebula* methanolic extract). The *T. catappa* hexane extract was also a potent *S. pyogenes* growth inhibitor (MIC 768 µg/mL). All other extracts were either ineffective or were of only low efficacy. Furthermore, all of the Indian *Terminalia*

spp. extracts were nontoxic in the *Artemia franciscana* bioassay, with LC<sub>50</sub> values > 1000 µg/mL. **Conclusion:** The potent growth inhibitory bioactivity of the methanolic and ethyl acetate *T. arjuna*, *T. catappa* and *T. chebula* extracts against *S. pyogenes* demonstrates their potential for the treatment and prevention of pharyngitis, impetigo and rheumatic heart disease. All extracts were nontoxic indicating their safety for therapeutic use.

**Key words:** *Terminalia arjuna*, *Terminalia catappa*, *Terminalia chebula* pharyngitis, Impetigo, Rheumatic heart disease, Antibacterial activity, Ayurveda.

## Correspondence:

Ian Edwin Cock,

School of Natural Sciences, Nathan Campus,  
Griffith University, 170 Kessels Rd, Nathan,  
Queensland 4111, AUSTRALIA.

Tel no.: +61 7 37357637; Fax no: +61 737355282.

**E-mail:** i.cock@griffith.edu.au (I. E. Cock)

**DOI :** 10.5530/pc.2016.2.6

## INTRODUCTION

The genus *Streptococcus* comprises over 50 different species of gram-positive, non-sporulating cocci-shaped bacteria. Found in a diverse range of environments including soils, or as part of the natural human microflora, *Streptococcus* spp. are primarily facultatively anaerobic, however some are obligate anaerobes.<sup>1</sup> Many species within the genus are pathogenic and responsible for an extensive variety of diseases. Pathogens within the genus can infect ruminants, humans or cause disease in both people and animals.<sup>2</sup> Diseases in humans can vary and range from non-life threatening epithelial/throat infections, such as pharyngitis or skin infections including impetigo and scarlet fever, to potentially fatal internal infections such as pneumonia, necrotizing fasciitis, toxic shock syndrome or meningitis.<sup>3,4</sup> Some *Streptococcus* species can also trigger autoimmune rheumatic heart disease.<sup>5</sup>

*Streptococcus* spp. are grouped according to their haemolytic properties and Lancefield serotyping. Group A streptococcal pharyngitis is an acute infection of the nasopharynx and/or oropharynx and is initiated through infection by *Streptococcus pyogenes*.<sup>6</sup> *S. pyogenes* infections are the most common bacterial cause of pharyngitis and are responsible for up to 33% of all diagnosed cases of sore throat in children, and up to 10% in adults.<sup>7</sup> Though mostly non-life threatening, Group A streptococcal infections are a significant economic burden. Indeed, recent estimates place the societal cost (both medical and nonmedical) in the United States alone ranging from \$224 to \$539 million dollars annually.<sup>8</sup> While the bacterium

responds well to antibiotic treatment,<sup>7</sup> the increasing risk of drug resistance highlights the need to develop alternatives to fight these and other diseases. The probing of natural plant resources for previously undiscovered antibacterial products offers an alternative to the traditional drug design and synthesis.

One of the most useful genera of therapeutic plants is *Terminalia*. This genus encompasses approximately 200-250 species of flowering trees and has extensive uses in multiple traditional medicinal systems.<sup>9</sup> The antibacterial activity of *Terminalia* spp. has been particularly well reported. Extracts prepared from the fruit of the Australian species *Terminalia ferdinandiana* (Kakadu plum) have potent growth inhibitory activity against an extensive panel of pathogens including bacteria associated with diarrhoea and dysentery<sup>10</sup> as well as the bacterial triggers of rheumatoid arthritis (*Proteus mirabilis*)<sup>11</sup> and multiple sclerosis (*Acinetobacter baylyi* and *Pseudomonas aeruginosa*).<sup>12</sup> Leaf extracts from the same species have also been shown to inhibit growth of the same bacteria, as well as a microbial trigger of ankylosing spondylitis (*Klebsiella pneumoniae*).<sup>13</sup> Similarly, African *Terminalia* spp. have been shown to be potent bacterial growth inhibitors. *Terminalia stenostachya* and *Terminalia spinosa* have strong antibacterial activity against a broad spectrum of medically important bacteria including several *Mycobacterium* spp., *Streptococcus faecalis*, *Staphylococcus aureus*, *Vibrio cholera*, *Bacillus anthracis*, *K. pneumoniae*, *Salmonella typhi*, *P. aeruginosa* and *Escherichia coli*.<sup>14</sup>

Recent studies have demonstrated the growth inhibitory activity of *Terminalia sericea* and *Terminalia pruinoides* against pathogenic<sup>15-17</sup> and food spoilage bacteria.<sup>18</sup>

The traditional therapeutic uses of the Indian *Terminalia* have been the best documented of all *Terminalia* spp. due to their usage in multiple medicinal systems, including Ayurveda, Siddha and Unani. Many species are used to treat multiple diseases caused by microbial infections (Table 1). Numerous recent investigations have reported on their antibacterial properties. Leaf and branch extracts of *Terminalia arjuna*, have antibacterial activity against a wide panel of microbes.<sup>19,20</sup> *Terminalia chebula* has traditional uses in Ayurveda for the treatment of numerous diseases and conditions<sup>21</sup> and has potent antibacterial activity.<sup>19</sup> Indeed, a recent study has even highlighted their potential in the prevention and treatment of anthrax.<sup>22</sup> *Terminalia alata*, *Terminalia bellirica* and *Terminalia catappa* also have broad spectrum antibacterial activity.<sup>20</sup> However, despite the wealth of antibacterial studies for *Terminalia* spp., there is a lack of studies screening *Terminalia* spp. for the ability to inhibit *S. pyogenes* growth and thus their therapeutic value for the prevention and treatment of streptococcal pharyngitis, impetigo, rheumatic heart disease etc. Indeed, a literature search only found a single study which reported *S. pyogenes* growth inhibitory activity for the Indian *Terminalia* spp. *T. chebula*.<sup>23</sup> However, whilst anti-*Streptococcal* activity was reported, the value of that study is limited as the extracts were screened only with a single, relatively high extract concentration. Our study was undertaken to examine the ability of selected Asian *Terminalia* spp. with extensive usage in Ayurvedic medicine for the ability to inhibit *S. pyogenes* growth.

## MATERIALS AND METHODS

### Plant source and extraction

*Terminalia arjuna*, *Terminalia catappa* and *Terminalia chebula* plant materials used throughout this study were kindly provided by Dr. Paran Rayan, Griffith University. Voucher samples of all plant specimens have been stored at the School of Natural Sciences, Griffith University, Brisbane Australia. The plant materials were comprehensively desiccated in a Sunbeam food dehydrator and the dried materials kept at -30°C until use. Prior to usage, the materials were thawed and ground into a coarse powder. Individual 1 g amounts of the material were weighed into separate tubes and 50 mL of deionised water, methanol, chloroform, hexane and ethyl acetate were added. All solvents were AR grade and were obtained from Ajax, Australia. The ground plant materials were individually extracted in each solvent for 24 h at 4°C using gentle shaking. The extracts were then filtered through filter paper (Whatman No. 54) under vacuum, followed by drying by rotary evaporation in an Eppendorf concentrator 5301. The resultant extracts were weighed and redissolved in 10 mL deionised water (containing 1 % DMSO).

### Qualitative phytochemical studies

Phytochemical analysis of the extracts for the presence of triterpenoids, tannins, saponins, phytosteroids, phenolic compounds, flavonoids, cardiac glycosides, anthraquinones and alkaloids was performed as previously described.<sup>24-26</sup>

### Antibacterial screening

#### *Clinical Streptococcus pyogenes* strain

All media was supplied by Oxoid Ltd., Australia. The clinical isolate strain of *Streptococcus pyogenes* used in this study was donated by Michelle Mendell of the School of Natural Sciences Griffith University, Australia. All growth studies were performed using nutrient agar (Oxoid Ltd., Australia) under aerobic conditions. Incubation was at 37°C and the stock culture was subcultured and maintained in nutrient broth at 4°C.

### Evaluation of antimicrobial activity

The antimicrobial activity of the *T. arjuna*, *T. chebula* and *T. catappa* plant extracts was assessed using a modified disc diffusion assay as previously described.<sup>16,27,28</sup> Briefly, 100 µL of *S. pyogenes* was grown in 10 mL of fresh nutrient broth until a cell count of ~10<sup>8</sup> cells/mL was attained. A 100 µL volume of bacterial suspension was spread onto nutrient agar plates. The antibacterial activity of the extracts was tested using 5 mm sterilised filter paper discs. Discs were infused with 10 µL of the each individual extract, allowed to dry and placed onto inoculated plates. The plates were allowed to stand at 4°C for 2 h before incubation at 30°C for 24 h. The diameters of the inhibition zones were measured in millimetres. All measurements were rounded to the closest whole millimetre. Each assay was performed in at least triplicate. Mean values (± SEM) are reported in this study. Standard discs of ampicillin (10 µg) were obtained from Oxoid Ltd. and served as positive controls for antibacterial activity. Filter discs infused with 10 µL of sterilised water were used as a negative control.

### Minimum inhibitory concentration (MIC) determination

The minimum inhibitory concentration (MIC) of each extract was assessed as previously described.<sup>29,30</sup> Briefly, the plant extracts were diluted in deionised water and tested across a range of concentrations. Discs were infused with 10 µL of the test dilutions, allowed to dry and placed onto inoculated plates. The assay was performed as outlined above and graphs of the zone of inhibition versus concentration were plotted for each extract. Linear regression was used to determine the MIC values of each extract.

### Toxicity screening

#### Reference toxin for toxicity screening

Potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) (AR grade, Chem-Supply, Australia) was prepared as a 4 mg/mL solution in distilled water and was serially diluted in artificial seawater for use in the *Artemia franciscana* nauplii bioassay.

#### *Artemia franciscana* nauplii toxicity screening

Toxicity was tested using an adapted *Artemia franciscana* nauplii lethality assay as previously described.<sup>31-33</sup> Briefly, 400 µL of seawater containing ~52 (mean=52.3, n=120, SEM=12.6) *A. franciscana* nauplii were added to wells of a 48 well plate and immediately used for bioassay. A volume of 400 µL of diluted plant extracts or the reference toxin were transferred to the wells and incubated at 25 ± 1°C under artificial light (1000 Lux). A negative control (400 µL seawater) was run in triplicate for each plate. All treatments were performed in at least triplicate. The wells were checked at regular intervals and the number of dead counted. The nauplii were deemed dead if no movement of the appendages was detected within 10 sec. Following 24 h exposure, all nauplii were sacrificed and counted to determine the total % mortality per well. The LC<sub>50</sub> with 95% confidence limits for each treatment was assessed using probit analysis.

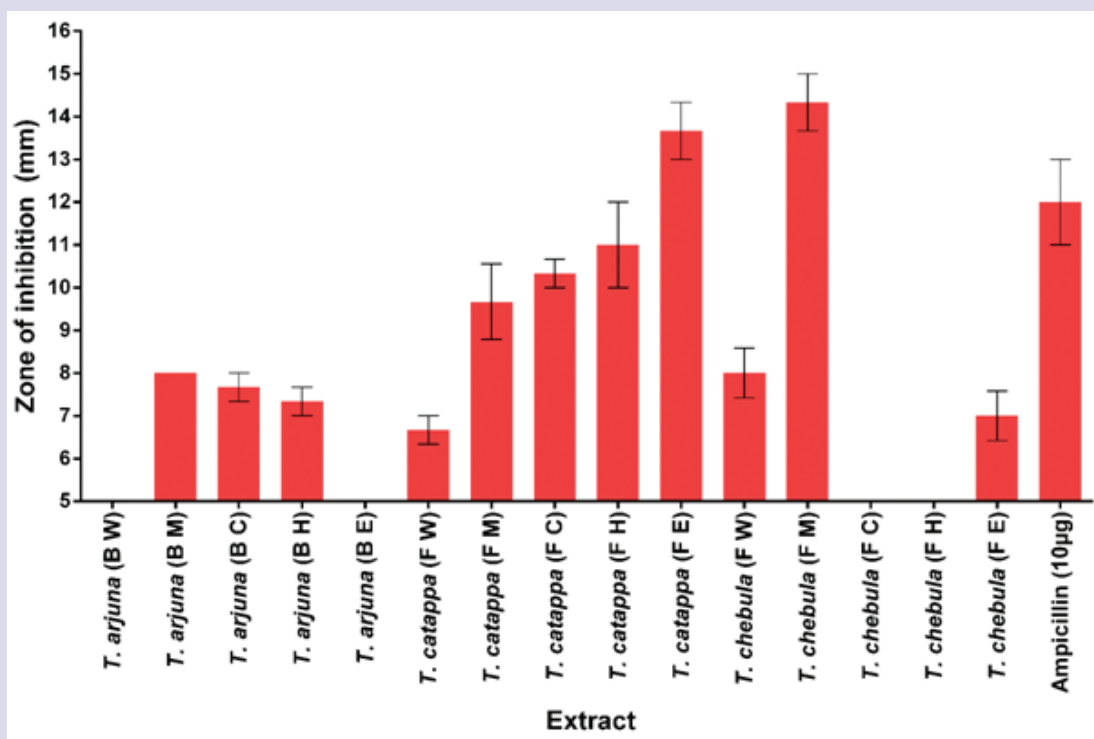
### Statistical analysis

Data are expressed as the mean ± SEM of at least three independent experiments.

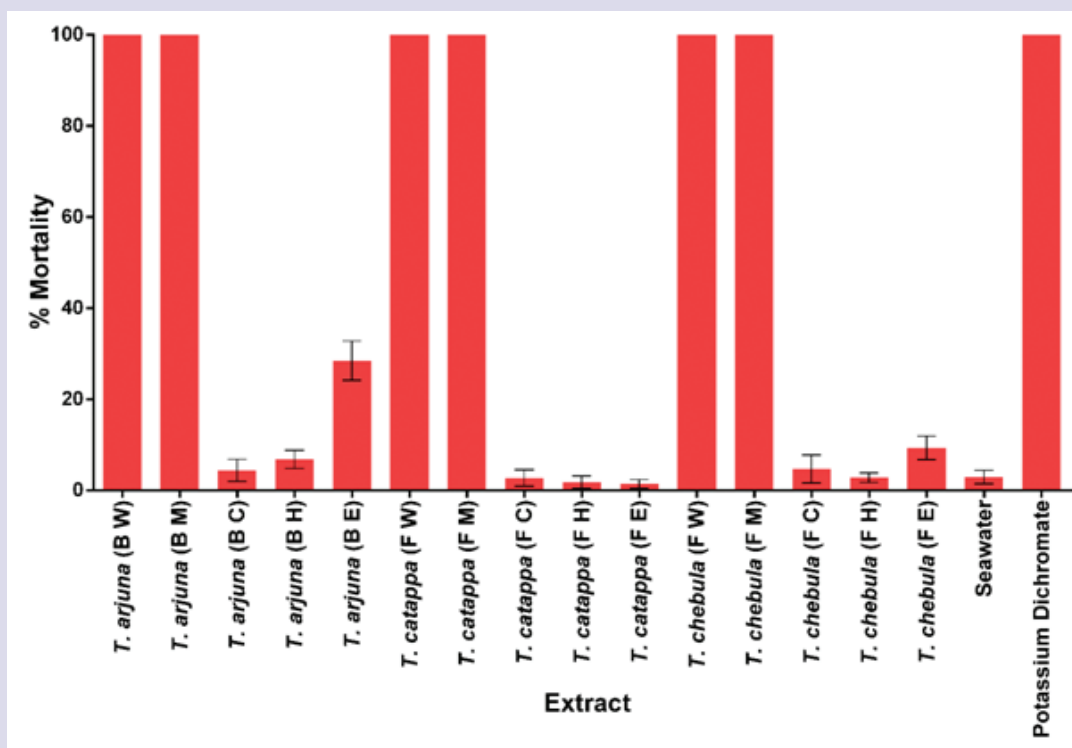
## RESULTS

### Liquid extraction yields and qualitative phytochemical screening

Extraction of 1 g of the various dried *Terminalia* spp. materials with the solvents yielded dried plant extracts ranging from 22 mg (*T. arjuna*



**Figure 1:** Growth inhibitory activity of the *Terminalia* spp. extracts against the *S. pyogenes* clinical isolate measured as zones of inhibition (mm). B=branch; F=fruit; W=aqueous extract; M=methanolic extract; C=chloroform extract; H=hexane extract; E=ethyl acetate extract. Results are expressed as mean zones of inhibition  $\pm$  SEM



**Figure 2:** The lethality of the *Terminalia* spp. extracts (2000 µg/mL) and the potassium dichromate (1000 µg/mL) and seawater controls towards *Artemia franciscana* nauplii after 24 h exposure. F=fruit; B=branch; W=aqueous extract; M=methanolic extract; C=chloroform extract; H=hexane extract; E=ethyl acetate extract. Results are expressed as mean % mortality  $\pm$  SEM.

**Table 1: The medicinal usage, common names and known constituents of the Indian *Terminalia* species tested in this study**

Plant Species	Part Used in This Study	Common Name/s	Traditional Medicinal Uses	Known Constituents	References
<i>Terminalia arjuna</i>	branch	Arjuna, Koha, White Marudah	Treatment of cardiovascular disorders as well as anti-inflammatory properties. Known to aid in the elimination of cholesterol. Also an analgesic and an antioxidant.	Triterpenoids, flavonoids, tannins, gallic and ellagic acid, sitosterol, proanthocyanidins	9
<i>Terminalia catappa</i>	fruit	Indian almond, tropical almond, umbrella tree	Therapeutic effects for liver related diseases, anticancer activity as well as effective in the blocking of HIV reverse transcriptase. Additionally known to have antidiabetic benefits.	Flavonoids (including kaempferol, quercetin), tannins, saponins and phytosterols	9
<i>Terminalia chebula</i>	fruit	Chebolic Myroblan, Black Myroblan, Haritaki, Inknut	Used externally to treat fungal infections and cutaneous wounds and in the prevention of inflammation of the mucosal membrane of the mouth. Used internally as a laxative and is known for its purgative effects. Known to have uses in the treatment of asthma and coughs.	Terflavin B and chebulinic acid	9

**Table 2: The mass of dried extracted material, the concentration after resuspension in deionised water and qualitative phytochemical screenings of the plant extracts**

Species	Plant Part Used	Extract	Mass of Dried Extract (mg)	Concentration of Resuspended Extract (mg/ml)	Total Phenolics	Water Soluble Phenolics	Water Insoluble Phenolics	Cardiac Glycosides	Saponins	Triterpenes	Polysteroids	Alkaloids (Mayer Test)	Alkaloids (Wagner Test)	Flavonoids	Tannins	Free Anthraquinones	Combined Anthraquinones
<i>T. arjuna</i>	B	W	144	14.4	++	+++	+++	-	++	-	-	-	-	+++	+++	+	-
<i>T. arjuna</i>	B	M	40	4	++	+++	+++	-	+++	-	-	-	-	+++	+++	+	-
<i>T. arjuna</i>	B	C	92	9.2	+	+	-	-	-	-	-	-	-	-	+	-	-
<i>T. arjuna</i>	B	H	136	13.6	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>T. arjuna</i>	B	E	22	2.2	+	-	-	-	-	-	-	-	-	+	+	-	-
<i>T. catappa</i>	F	W	144	14.4	+++	+++	++	+	++	-	-	++	++	++	+++	-	-
<i>T. catappa</i>	F	M	231	23.1	+++	+++	++	+	-	-	-	+	++	++	+++	-	-
<i>T. catappa</i>	F	C	434	43.4	+	+	-	+	-	-	-	-	-	+	+	-	-
<i>T. catappa</i>	F	H	447	44.7	+	+	-	-	-	-	-	-	-	-	+	-	-
<i>T. catappa</i>	F	E	353	35.3	+	+	-	-	-	-	-	-	-	+	+	-	-
<i>T. chebula</i>	F	W	438	43.8	+++	+++	+++	-	+++	-	-	-	-	+++	+++	++	-
<i>T. chebula</i>	F	M	634	63.4	+++	+++	+++	-	+++	-	-	-	-	+++	+++	++	-
<i>T. chebula</i>	F	C	93	9.3	+	-	-	-	-	-	-	-	-	-	+	-	-
<i>T. chebula</i>	F	H	104	10.4	-	-	-	-	-	-	-	-	-	-	-	++	-
<i>T. chebula</i>	F	E	62	6.2	+++	++	+	-	-	-	-	-	-	+++	+++	-	-

+++ indicates a large response; ++ indicates a moderate response; + indicates a minor response; - indicates no response in the assay. F=fruit; B=branch; W=aqueous extract; M=methanolic extract; C=chloroform extract; H=hexane extract; E=ethyl acetate extract.

ethyl acetate leaf extract) to 634 mg (*T. chebula* methanolic fruit extract) (Table 2). Typically, *T. chebula* fruit extracts gave higher yields compared to *T. arjuna* and *T. catappa* extracted materials. However, this trend was not observed in the lower polarity fruit extracts (hexane and chloroform) obtained from *T. catappa*, which had significantly higher yields compared to those of *T. arjuna* and *T. chebula*. Indeed, the *T. catappa* hexane and chloroform extracts had approximately double the yields when compared to the corresponding methanolic or aqueous extracts.

Qualitative phytochemical studies showed that the methanolic and aqueous extracts generally had an extensive range of phytochemicals (Table 2). Both these solvents typically extracted greater levels of phenolics (especially water soluble phenolics) for all plant materials. Additionally, these extracts generally yielded high levels of tannins and flavonoids and moderate to high levels of saponins. Similarly, the ethyl acetate extracts had similar phytochemical profiles to the aqueous and methanolic counterparts. However, most classes of compounds were present in only lower relative abundances. Conversely, the chloroform and hexane extracts of most of the *Terminalia* spp. typically only had low to moderate levels of phenolics, flavonoids and tannins and were generally devoid of detectable levels of the other phytochemical classes.

### Antimicrobial activity

To assess the inhibitory activity of the crude plant extracts against *S. pyogenes*, 10 µL aliquots of each extract were screened using a disc diffusion assay. The bacterial growth was inhibited by 11 of the 15 extracts tested (~73%) (Figure 1). The *T. catappa* ethyl acetate and *T. chebula* methanolic extracts were the most potent inhibitors of *S. pyogenes* growth (as judged by zones of inhibition), with inhibition zones of  $13.6 \pm 0.7$  mm and  $14.3 \pm 0.7$  mm respectively. This compares favourably with the ampicillin control, which had an inhibitory zone of  $12.0 \pm 1.0$  mm. Collectively, *T. catappa* extracts were the best inhibitors of growth, with all extracts testing positive and the methanolic, chloroform and hexane extracts giving > 9 mm inhibitory zones.

The antimicrobial efficacy was further quantified by determining the MIC values (Table 3). In general, the ethyl acetate extracts were the most potent inhibitors of *S. pyogenes* growth, with MIC values of approximately 200 µg/mL (~2 µg infused into the disc). The *T. arjuna* ethyl acetate extract defied this trend with no growth inhibitory activity evident for this extract at all. However, it is noteworthy that this extract was the least concentrated of the tested extracts and it is possible it may have exhibited activity if tested at a higher concentration. Similarly potent *S. pyogenes* growth inhibition was also noted for the methanolic extracts of all *Terminalia* spp. (MIC values 250–400 µg/mL). With the exceptions of the *T. arjuna* chloroform and hexane extracts, the *T. catappa* hexane extract and the aqueous *T. chebula* extract (which displayed moderate growth inhibitory activity), all other extracts were ineffective at inhibiting *S. pyogenes* growth.

### Quantification of toxicity

All extracts were initially screened at 2000 µg/mL in the assay (Figure 2). For comparison, the reference toxin potassium dichromate (1000 µg/mL) was also assessed in the bioassay. The potassium dichromate reference toxin was rapid in its onset, inducing nauplii death within the first 3 h of exposure and 100% mortality evident in the subsequent 4–5 h (unpublished results). The methanolic and aqueous extracts of all *Terminalia* spp. were toxic in the *Artemia* nauplii bioassay, with ≥50% mortality rates at 24 h. The mortality for all other extracts was not significantly different to the mortality seen for the seawater control.

To further enumerate the effect of toxin concentration on the induction of mortality, the extracts were serially diluted in artificial seawater to test across a range of concentrations in the *Artemia* nauplii bioassay.

Table 3 shows the LC<sub>50</sub> values of the extracts towards *A. franciscana*. No LC<sub>50</sub> values are reported for the ethyl acetate, chloroform or hexane extracts of any *Terminalia* spp. as ≤50% mortality was seen across concentrations tested. All other extracts were deemed nontoxic as they yielded LC<sub>50</sub> values substantially greater than 1000 µg/mL following 24 h exposure. Extracts with LC<sub>50</sub> values of ≥1000 µg/mL towards *Artemia* nauplii are deemed to be nontoxic.<sup>33</sup>

**Table 3: Minimum inhibitory concentration (µg/mL) of the plant extracts and LC<sub>50</sub> values (µg/mL) in the *Artemia* nauplii bioassay**

Species	Part	Extract	MIC (µg/mL)	LC <sub>50</sub> (µg/mL)
<i>T. arjuna</i>	B	W	-	2094
<i>T. arjuna</i>	B	M	268	1683
<i>T. arjuna</i>	B	C	2581	-
<i>T. arjuna</i>	B	H	3300	-
<i>T. arjuna</i>	B	E	-	-
<i>T. catappa</i>	F	W	>10,000	1873
<i>T. catappa</i>	F	M	425	1452
<i>T. catappa</i>	F	C	>10,000	-
<i>T. catappa</i>	F	H	768	-
<i>T. catappa</i>	F	E	225.1	-
<i>T. chebula</i>	F	W	2677	2246
<i>T. chebula</i>	F	M	300	1883
<i>T. chebula</i>	F	C	-	-
<i>T. chebula</i>	F	H	-	-
<i>T. chebula</i>	F	E	205	-

Numbers indicate the mean MIC and LC<sub>50</sub> values of triplicate determinations. - indicates no bacterial growth inhibition was evident, or that an LC<sub>50</sub> value could not be obtained as the mortality did not reach 50% for any dose tested. F=fruit; B=branch; W=aqueous extract; M=methanolic extract; C=chloroform extract; H=hexane extract; E=ethyl acetate extract.

## DISCUSSION

Members of genus *Terminalia* have been used for a broad range of medicinal purposes by traditional healers from a wide variety of ethnic and cultural groupings. The best documented of these are the traditional Indian medicinal systems, particularly the Ayurveda. Ayurvedic practitioners employ various *Terminalia* spp. for a wide variety of medicinal purposes including abdominal and back pain, coughs and colds, conjunctivitis, diarrhoea and dysentery, fever, headache, heart disorders, inflammation, leprosy, pneumoniae, sexually transmitted diseases, worms, wounds, haemorrhages, ulcers, and as a general tonic.<sup>9</sup> Many of these diseases are caused by microbial pathogens, indicating the potential of these plants as antiseptic agents and numerous recent investigations have reported on their antibacterial properties. *T. arjuna* leaf and branch extracts have antibacterial activity against a wide panel of microbes.<sup>9,19,20</sup> *T. chebula* also has a tradition of use in Ayurveda for the treatment of numerous diseases and conditions.<sup>9,21</sup> *T. chebula* has also been reported to display potent antibacterial activity against a microbial panel.<sup>19</sup> Similarly, *T. alata*, *T. bellirica* and *T. catappa* have been reported to have broad spectrum antibacterial activity.<sup>20</sup>

The growth inhibitory activity of the Indian *Terminalia* spp. extracts against *S. pyogenes* is particularly noteworthy for the development of

future antibiotic chemotherapeutics. Aside from the obvious antibiotic applications to directly treat localised throat (pharyngitis) and skin infections (impetigo),<sup>6,7</sup> a number of substantially more serious illnesses are caused by acute and chronic *S. pyogenes* infections and may also benefit from treatment with these extracts. When *S. pyogenes* invades and colonises deeper tissue it can lead to erysipelas and cellulitis, conditions characterised by localised red, swollen and painful areas, and often by fever and lethargy.<sup>2-4</sup> If not promptly treated, bacterium can spread to other areas via the bloodstream which may result in serious tissue damage and autoimmune diseases such as glomerulonephritis (inflammation of the glomeruli in the kidneys), lymphedema (inflammation of lymph nodes), septic arthritis and rheumatic fever (inflammation of cardiac tissue).<sup>3-5</sup> Furthermore, acute *S. pyogenes* infections of subcutaneous tissues can induce the potentially fatal disease necrotizing fasciitis.<sup>4</sup> These conditions are not only highly debilitating, but may also be life threatening and new, more effective treatment regimens could potentially prolong and increase the quality of life as well as reducing the burden on the health system. The efficacy of the Indian *Terminalia* spp. extracts indicates that they may have potential in the treatment of these illnesses and further investigation is warranted.

Whilst an examination of the phytochemistry of the *Terminalia* spp. was beyond the scope of our study, a commonality of this genus is their relatively high levels of a number of tannin components including exifone (4-galloylpyrogallol), ellagic acid dehydrate, trimethyl ellagic acid, chebulic acid, corilagen, castalagin and chebulagic acid.<sup>9,11-13</sup> Gallo-tannins have been reported to inhibit the growth of a broad spectrum of bacterial species<sup>34</sup> through a variety of mechanisms including binding cell surface molecules including lipoteichoic acid and proline-rich cell surface proteins,<sup>35,36</sup> and by inhibiting glucosyltransferase enzymes.<sup>37</sup> Ellagitannins are also highly potent inhibitors of bacterial growth, with MIC values as low as 62.5 µg/ml.<sup>34</sup> Ellagitannins have also been reported to function via several antibiotic mechanisms including interaction with cytoplasmic oxidoreductases and by disrupting bacterial cell walls.<sup>34,36</sup>

It is likely that other phytochemical classes also contribute to the growth inhibitory properties of these extracts. Our qualitative phytochemical screening studies indicate that polyphenolics, flavonoids, saponins, and terpenes were present in the *Terminalia* spp. extracts. Terpenoids have been previously reported to have potent broad spectrum antibacterial activity<sup>38</sup> and therefore may contribute to the inhibitory activity against *S. pyogenes*. Many studies have also reported potent antibacterial activities for a wide variety of flavonoids.<sup>39</sup> Further phytochemical evaluation studies and bioactivity driven isolation of active components is required to further evaluate the mechanism of *S. pyogenes* growth inhibition.

The findings reported here also demonstrate that all of the Indian *Terminalia* spp. extracts tested in our study were nontoxic towards *Artemia franciscana* nauplii, with LC<sub>50</sub> values substantially >1000 µg/mL. Extracts with LC<sub>50</sub> values >1000 µg/mL towards *Artemia* nauplii are defined as being nontoxic.<sup>33</sup> Whilst our preliminary toxicity studies indicate that these extracts may be safe for use as *S. pyogenes* growth inhibitors, studies using human cell lines are required to further evaluate the safety of these extracts.

## ACKNOWLEDGEMENTS

We are grateful to Dr Paran Rayan for supplying the plant specimens and to Michelle Mendell for the gift of the *S. pyogenes* clinical isolate strain used in this study. Financial support for this work was provided by the Environmental Futures Research Institute and the School of Natural Sciences, Griffith University, Australia.

## CONFLICTS OF INTEREST

The authors report no conflicts of interest.

## ABBREVIATION USED

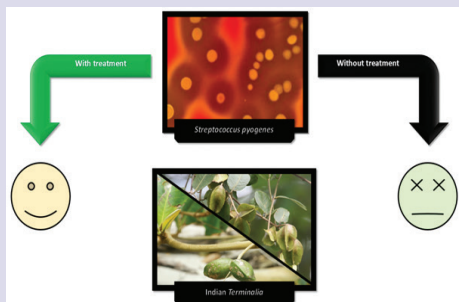
**DMSO:** Dimethyl sulfoxide, **LC<sub>50</sub>:** The concentration required to achieve 50% mortality, **MIC:** Minimum inhibitory concentration.

## REFERENCES

- Patterson MJ. Streptococcus. In: Baron S, editor. Medical Microbiology. 4th edition. Chapter 13. Galveston (TX): University of Texas Medical Branch at Galveston 1996. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK7611/>
- Wertheim HFL, Nghia HDT, Taylor W. Streptococcus suis: An Emerging Human Pathogen. Clin Infect Dis. 2009;48(5):617-25.
- O'Brien KL, Wolfson LJ, Watt JP. Burden of disease caused by Streptococcus pneumoniae in children younger than 5 years: global estimates. The Lancet. 2009;374(9693):893-902.
- Johansson L, Thulin P, Low DE. Getting under the skin: The Immunopathogenesis of Streptococcus pyogenes deep tissue infections. Clinical Infectious Diseases. 2010;51(1):58-65.
- Gerber MA, Baltimore RS, Eaton CB. Prevention of rheumatic fever and diagnosis and treatment of acute Streptococcal pharyngitis. A scientific statement from the American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee of the Council on Cardiovascular Disease in the Young, the Interdisciplinary Council on Functional Genomics and Translational Biology, and the Interdisciplinary Council on Quality of Care and Outcomes Research: Endorsed by the American Academy of Pediatrics. Circulation. 2009;119(11):1541-51.
- Bisno AL, Gerber MA, Gwaltney JM. Practice Guidelines for the Diagnosis and Management of Group A Streptococcal Pharyngitis. Clinical Infectious Diseases. 2002;35(2):113-25.
- Worrall GJ. Acute sore throat. Canadian Family Physician. 2007;53(11):1961-2.
- Pfoh E, Wessels MR, Goldmann D. Burden and economic cost of group A streptococcal pharyngitis. Pediatrics. 2008;121(2):229-34.
- Cock IE. The medicinal properties and phytochemistry of plants of the genus Terminalia (Combretaceae). Inflammopharmacology. 2015;23(5):203-29. DOI 10.1007/s10787-015-0246-z
- Cock IE, Mohanty S. Evaluation of the antibacterial activity and toxicity of Terminalia ferdinandiana fruit extracts. Pharmacognosy Journal. 2011;3(20):72-9. DOI: 10.5530/pj.2011.20.14
- Sirdaarta J, Matthews B, Cock IE. Kakadu plum fruit extracts inhibit growth of the bacterial triggers of rheumatoid arthritis: Identification of stilbene and tannin components. Journal of Functional Foods. 2015;17:610-20. DOI: 10.1016/j.jff.2015.06.019
- Sirdaarta J, Matthews B, White A, et al. GC-MS and LC-MS analysis of Kakadu plum fruit extracts displaying inhibitory activity against microbial triggers of multiple sclerosis. Pharmacognosy Communications. 2015;5(2):100-15. DOI: 10.5530/pc.2015.2.2
- Courtney R, Sirdaarta J, Matthews B, et al. Tannin components and inhibitory activity of Kakadu plum leaf extracts against microbial triggers of autoimmune inflammatory diseases. Pharmacognosy Journal. 2015;7(1):18-31. DOI: 10.5530/pj.2015.7.2
- Mbwambo ZH, Erasto P, Nondo RO. Antibacterial and cytotoxic activities of Terminalia stenostachya and Terminalia spinosa. Tanzania Journal of Health Research. 2011;13(2):1-8.
- Cock IE, van Vuuren SF. Anti-Proteus activity of some South African medicinal plants: Their potential for the prevention of rheumatoid arthritis. Inflammopharmacology. 2014;22(1):23-36. DOI 10.1007/s10787-013-0179-3
- Cock IE, van Vuuren SF. The potential of selected South African plants with anti-Klebsiella activity for the treatment and prevention of ankylosing spondylitis. Inflammopharmacology. 2015;23(1):21-35. DOI: 10.1007/s10787-014-0222-z
- Cock IE, van Vuuren SF. A comparison of the antimicrobial activity and toxicity of six Combretum and two Terminalia species from Southern Africa. Pharmacognosy Magazine. 2015;11(4):208-18. DOI 10.4103/0973-1296.149740
- Cock IE, van Vuuren SF. South African food and medicinal plant extracts as potential antimicrobial agents. Journal of Food Science and Technology. 2015;52(11):6879-99. DOI: 10.1007/s13197-015-1806-3
- Aneja KR, Sharma C, Joshi R. Antimicrobial activity of Terminalia arjuna Wright and Arn: An ethnomedicinal plant against pathogens causing ear infection. Brazilian Journal of Otorhinolaryngology. 2012;78(1):68-74.
- Shinde SL, Junne SB, Wadje SS, Baig MMV. The diversity of antibacterial compounds of Terminalia species (Combretaceae). Pakistan Journal of Biological Sciences. 2009;12(22):1483-6.
- Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants. New Delhi, CSIR, 1956.
- Wright MH, Courtney R, Greene AC, et al. Growth inhibitory activity of Indian Terminalia spp. against the zoonotic bacterium Bacillus anthracis. Pharmacognosy Communications. 2016;6(1):2-9. DOI: 10.5530/pc2016.1.2.
- Kim HG, Cho JH, Jeong EY. Growth-inhibiting activity of active component isolated from Terminalia chebula fruits against intestinal bacteria. Journal of Food Protection. 2006;69(9):2205-9.

24. Arkhipov A, Sirdaarta J, Rayan P, *et al.* An examination of the antibacterial, anti-fungal, anti-Giardial and anticancer properties of *Kigelia africana* fruit extracts. *Pharmacognosy Communications*. 2014;4(3):62-76. DOI: 10.5530/pc.2014.3.7
25. Kalt FR, Cock IE. Gas chromatography-mass spectroscopy analysis of bioactive *Petalostigma* extracts: Toxicity, antibacterial and antiviral activities. *Pharmacognosy Magazine*. 2014;10(Suppl 1):S37-49. DOI: 10.4103/0973-1296.127338
26. Vesoul J, Cock IE. The potential of Bunya Nut as an antibacterial food agent. *Pharmacognosy Communications*. 2012; 2 (1):72-9. DOI: 10.5530/pc.2012.1.13
27. Cock IE, Winnett V, Sirdaarta J, *et al.* The potential of selected Australian medicinal plants with anti-Proteus activity for the treatment and prevention of rheumatoid arthritis. *Pharmacognosy Magazine*. 2015;42(Suppl 1):S190-S208. DOI: 10.4103/0973-1296.157734
28. Sautron C, Cock IE. Antimicrobial activity and toxicity of *Syzygium australe* and *Syzygium luehmannii* fruit extracts. *Pharmacognosy Communications*. 2014;4(1):53-60. DOI: 10.5530/pc.2014.1.8
29. Wright MH, Matthews B, Greene AC, *et al.* Growth inhibition of the zoonotic bacteria *Bacillus anthracis* by high antioxidant Australian plants: Newleads for the prevention and treatment of anthrax. *Pharmacognosy Communications*. 2015; 5(3):173-89. DOI: 10.5530/pc.2015.3.3
30. Boyer H, Cock IE. Evaluation of the potential of *Macadamia integriflora* extracts as antibacterial food agents. *Pharmacognosy Communications*. 2013;3(3):53-62. DOI: 10.5530/pc.2013.3.10
31. Cock IE. Assessment of the toxicity of selected Australian native plant extracts using the *Artemia franciscana* nauplii bioassay. *Internet Journal of Toxicology*. 2008; 5(2).
32. Ruebhart D, Wickramasinghe W, Cock IE. Protective efficacy of the antioxidants vitamin E and Trolox against *Microcystis aeruginosa* and microcystin-LR in *Artemia franciscana* nauplii. *Journal of Toxicology and Environmental Health, Part A*. 2009;72(24):1567-75. DOI: 10.1080/15287390903232459
33. Cock IE, Ruebhart DR. Comparison of the brine shrimp nauplii bioassay and the ToxScreen-II test for the detection of toxicity associated with Aloe vera (*Aloe barbadensis* Miller) leaf extract. *Pharmacognosy Research*. 2009;1(2):98-101.
34. Buzzini P, Arapitsas P, Goretti M. Antimicrobial activity of hydrolysable tannins. *Mini-Reviews in Medicinal Chemistry*. 2008;8:1179-87.
35. Wolinsky LE, Sote EO. Isolation of natural plaque-inhibiting substances from 'Nigerian chewing sticks'. *Caries Research*. 1984;18(3):216-25.
36. Hogg SD, Embery G. Blood-group-reactive glycoprotein from human saliva interacts with lipoteichoic acid on the surface of *Streptococcus sanguis* cells. *Archives in Oral Biology*. 1982;27(3):261-8.
37. Wu-Yuan CD, Chen CY, Wu RT. Gallotannins inhibit growth, water-soluble glucan synthesis, and aggregation of *Streptococci* mutans. *Journal of Dental Research*. 1988;67(1):51-5.
38. Cock IE. The phytochemistry and chemotherapeutic potential of *Tasmannia lanceolata* (Tasmanian pepper): A review. *Pharmacognosy Communications*. 2013;3(4):1-13. DOI: 10.5530/pc.2013.4.3
39. Narayana KR, Reddy MS, Chaluvadi MR. Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. *Indian Journal of Pharmacology*. 2001;33(1):2-16.

### PICTORIAL ABSTRACT



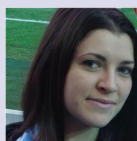
### SUMMARY

- Methanolic and ethyl acetate *T. arjuna*, *T. catappa* and *T. chebula* extracts were potent inhibitors of *S. pyogenes* growth.
- The *T. catappa* and *T. chebula* ethyl acetate extracts were particularly potent with MIC values of 225 and 205 µg/mL respectively.
- The methanolic extracts of all Indian *Terminalia* spp. were also potent growth inhibitors with MIC values 268-425 µg/mL.
- The *T. catappa* hexane extract was also a good *S. pyogenes* growth inhibitor (MIC 768 µg/mL)
- All other extracts were either ineffective *S. pyogenes* growth inhibitors or were of only low efficacy.
- All Indian *Terminalia* spp. extracts were nontoxic in the *Artemia* nauplii assay.

### ABOUT AUTHORS



**Dr. Mitchell Wright:** Received his PhD in 2014, for his work investigating the manganese reduction and oxidation characteristics of environmental bacteria. He is currently a postdoctoral researcher at Griffith University, Australia, where he is working on several projects both in the areas of geomicrobiology and pharmacognosy. His present research interests are the use of bacteriogenic manganese oxides in the bioremediation of metal-contaminated sites as well as the use of Australian native plants in the treatment and prevention of various pathogenic bacteria.



**Ms. Megan Arnold:** Is currently undertaking her PhD in Tropical Parasitology at Griffith University's Eskitis Institute for Drug Discovery with a focus on the identification and development of novel chemoprophylactic agents for malaria. Her other research interests include investigating Australian high antioxidant plants for their antibacterial capabilities.



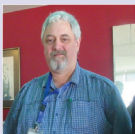
**Mr. Cameron Lee:** Completed his Bachelor of Science (BSc) in 2015 and is currently concluding his honours year. His research involves the investigation of thermophilic anaerobes that utilize toxic metals in anaerobic respiration (including uranium and arsenic). He has extensive experience in anaerobic cultivation/isolation and in numerous analytical techniques associated with heavy metal analysis.



**Mr. Reece Courtney:** Is a current postgraduate student in the School of Natural Sciences Griffith University, Australia under the supervision of Dr Ian Cock. His research interests include medical microbiology, pharmacognosy and medicinal plants. He has a particular interest in the medicinal properties of the genus *Terminalia*, which is the subject of his research project.



**Dr. Anthony Greene:** Is a senior lecturer and researcher at Griffith University, Brisbane Australia. He obtained his PhD in Microbiology from the University of New South Wales and focuses on extreme environments, Bioremediation and Geomicrobiology. His specific interests include the microbial ecology of thermophilic, saline and alkaliphilic environments and the mechanisms and industrial potential of extremophilic bacteria contained therein.



**Dr. Ian Cock:** Leads a research team in the Environmental Futures Research Institute and the School of Natural Sciences at Griffith University, Australia. His research involves bioactivity and phytochemical studies into a variety of plant species of both Australian and international origin, including *Aloe vera*, South Asian and South American tropical fruits, as well as Australia plants including *Scaevola spinescens*, *Pittosporum phylliraeoides*, *Terminalia ferdinandiana* (Kakadu plum), Australian *Acacias*, *Syzygiums*, *Petalostigmas* and *Xanthorrhoea johnsonii* (grass trees). This range of projects has resulted in nearly 200 publications in a variety of peer reviewed journals.